

WHAT IS CLAIMED IS:

Sup 217. A process for selectively amplifying nucleic acid sequences, comprising forming a mixture comprising: multiple single stranded non-circular oligonucleotide primers (P1), one or more amplification target circles (ATCs), a DNA polymerase and multiple deoxynucleoside triphosphates, under conditions wherein said ATC binds to more than one of said multiple P1 primers and wherein conditions promote replication of said amplification target circle by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products.

2. The process of claim 1 wherein said multiple primers are primers with specific sequences complementary to portions of an ATC.

3. The process of claim 1 wherein said multiple primers are random primers.

4. The process of claim 1 wherein said multiple primers comprise a mixture of random and specific primers.

5. The process of claim 1 wherein said multiple primers are within the range of 2 to 50 nucleotides in length.

6. The process of claim 1 wherein said multiple primers are within the range of 2 to 35 nucleotides in length.

7. The process of claim 1 wherein said multiple primers are within the range of 2 to 10 nucleotides in length.

8. The process of claim 1 wherein said multiple primers are hexamers.

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9. The process of claim 1 wherein said multiple primers are octamers.

10. The process of claim 1 wherein said multiple primers contain a  
5 region at the 5' end of said primers non-complementary to the ATC.

11. The process of claim 1 wherein said ATC is a single stranded DNA  
circle.

10 12. The process of claim 1 wherein said ATC is a duplex DNA circle  
having at least one nick.

13. The process of claim 1 wherein said ATC is a duplex DNA circle  
having no nicks.

15 14. The process of claim 1 wherein said ATC is a single stranded RNA  
circle.

15 15. The processes of claim 12 or claim 13 further comprising a  
20 denaturation step to separate the two strands of the duplex DNA circle.

25 16. The method of claim 15 where the amplification target circle is derived  
directly from a member selected from the group consisting of bacterial colony,  
bacteriophage, virus plaque, yeast colony, baculovirus plaque, and transiently  
transfected eukaryotic cells.

17. The method of claim 16 wherein said member has been lysed.

30 18. The method of claim 17 wherein lysis is achieved by treatment with an  
agent selected from the group consisting of heat, an enzyme, and an organic  
solvent.

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19. The method of claim 18 where said enzyme is selected from the group consisting of lysozyme, ~~he~~lase, glucylase, and xymolyase.

5 20. The process of claim 1 wherein said ATC is no larger than about 10,000 nucleotides in size.

21. The process of claim 1 wherein said ATC is larger than 10,000 nucleotides in size.

10 22. The process of claim 1 wherein said ATC is no larger than about 1,000 nucleotides in size.

15 23. The process of claim 1 wherein said ATC is no larger than about 100 nucleotides in size.

24. The method of claim 1 wherein the amplification target circle comprises a single stranded bacteriophage DNA, a double stranded DNA plasmid or other vector, or a clone derived from such a vector.

20 25. The method of claim 1 wherein the amplification target circle to be amplified is of unknown sequence composition.

25 26. The process of claim 1 wherein said dNTP is a member selected from the group consisting of dTTP, dCTP, dATP, dGTP, dUTP, a naturally occurring dNTP different from the foregoing, an analog of a dNTP, and a dNTP having a universal base.

Sub B 1 27. The process of claim 26 wherein at least one said dNTP is  
30 radiolabeled.

28. The process of claim 26 wherein at least one nucleotide renders the TS-DNA resistant to nuclease activity following incorporation thereof.

5 29. The process of claim 28 wherein said at least one nucleotide is a phosphorothioate nucleotide.

30. The process of claim 28 wherein said nuclease activity is due to an endonuclease.

10 31. The process of claim 28 wherein said nuclease activity is due to an exonuclease.

32. The process of claim 31 wherein said exonuclease activity is due to a polymerase having a 3'-5' exonuclease activity.

33. The process of claim 31 wherein said exonuclease activity is due to an added exonuclease enzyme.

20 34. The process of claim 28 wherein said nuclease activity is due to a contaminating nuclease.

35. The process of claim 28 wherein said at least one nucleotide is a modified nucleotide.

25 36. The process of claim 1 wherein at least one P1 primer is attached to a solid support.

30 37. The process of claim 36 wherein said solid support is made of glass or plastic.

38. The process of claim 1 wherein said multiple primers are selected from the group consisting of primers resistant to exonuclease activity, primers not resistant to exonuclease activity and a mixture of primers sensitive to exonuclease activity and resistant to exonuclease activity.

39. The process of claim 1 wherein said multiple primers are resistant to exonuclease activity and said target DNA is selected from the group consisting of linear DNA, genomic DNA and cDNA.

40. The process of claim 38 wherein said exonuclease activity is caused by an enzyme.

41. The process of claim 38 wherein said exonuclease activity is caused by a 3'-5'-exonuclease.

42. The process of claim 38 wherein said exonuclease activity is caused by a DNA polymerase having 3'-5'-exonuclease activity.

43. The process of claim 38 wherein said exonuclease activity is caused by a contaminating nuclease.

44. The process of claim 38 wherein each of said exonuclease-resistant primers contains at least one nucleotide making said primer resistant to exonuclease activity.

45. The process of claim 44 wherein said at least one nucleotide is a modified nucleotide.

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46. The process of claim 45 wherein said modified nucleotide is a 3'-terminal nucleotide.

5 47. The process of claim 46 wherein said modified nucleotide is a phosphorothioate nucleotide.

48. The process of claim 44 wherein each of said exonuclease-resistant primers contains at least two nucleotides making said primer resistant to exonuclease activity.

10 49. The process of claim 35 wherein said at least one nucleotide is located at other than the 3'-terminal position.

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15 50. The process of claim 49 wherein said 3'-terminal nucleotide of said primer can be removed by 3'-5'-exonuclease activity.

20 51. The process of claim 1 wherein said DNA polymerase is a DNA polymerase having 3',5'-exonuclease activity and is a member selected from the group consisting of bacteriophage  $\phi$ 29 DNA polymerase, Tts DNA polymerase, phage M2 DNA polymerase, VENT<sup>TM</sup> DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase holoenzyme, T7 native polymerase and Bst DNA polymerase.

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25 52. The process of claim 1 wherein said DNA polymerase is bacteriophage  $\phi$ 29 DNA polymerase.

30 53. The process of claim 1 wherein said DNA polymerase is bacteriophage  $\phi$ -29 DNA polymerase and said multiple primers are resistant to exonuclease activity.

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54. The process of claim 1 wherein said DNA polymerase is bacteriophage  $\phi$ 29 DNA polymerase wherein said multiple primers are resistant to exonuclease activity and said target DNA is selected from the group consisting of linear DNA, genomic DNA and cDNA.

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55. The process of claim 1 wherein said DNA polymerase does not exhibit 3',5'-exonuclease activity.

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10 56. The process of claim 55 wherein said DNA polymerase is selected from the group consisting of DNA polymerases lacking a 3'-5' exonuclease activity, such as Taq, Tfl, and Tth DNA polymerase, Eukaryotic DNA polymerase alpha, and DNA polymerases that have been modified to eliminate a 3'-5' exonuclease activity such as exo (-) versions of  $\phi$ 29 DNA polymerase, Klenow fragment, Vent and Pfu DNA polymerases.

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57. The process of claim 1 wherein said DNA polymerase is a reverse transcriptase.

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20 58. The process of claim 1 wherein said ATC is RNA and said DNA polymerase is a reverse transcriptase.

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25 59. The process of claims 38-56 wherein said multiple primers are a mixture of primers sensitive to exonuclease activity and resistant to exonuclease activity.

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60. The process of claims 38-56 wherein a linear DNA target is used instead of said ATC.

30 61. The process of claim 60 wherein said DNA polymerase is  $\phi$ 29 DNA polymerase.

62. A process for selectively amplifying nucleic acid sequences, comprising:

- 5 (a) mixing multiple single stranded non-circular oligonucleotide primers (P1) and one or more amplification target circles (ATC) under conditions wherein said ATC binds to one said multiple P1 primers to produce a primer-ATC sample mixture;
- 10 (b) adding a DNA polymerase and multiple deoxynucleoside triphosphates under conditions that promote replication of said amplification target circle by extension of the P1 primers to form multiple primary tandem sequence DNA (TS-DNA) products.

63. The method of claim 1 where at least one of the deoxyribonucleoside triphosphates comprises a readily detectable moiety.

- 15 64. The method of claim 63 where the detectable moiety is a fluorescent label.

20 65. A kit for amplifying DNA sequences comprising nuclease-resistant random primers, a DNA polymerase and one or more deoxyribonucleoside triphosphates.

- 25 66. A kit for amplifying DNA sequences comprising both nuclease-sensitive and nuclease-resistant random primers, a DNA polymerase and one or more deoxyribonucleoside triphosphates.

67. The kit of claims 65 and 66, wherein said DNA polymerase has 3'-5' exonuclease activity.

- 30 68. The kit of claims 65 and 66, wherein said DNA polymerase is  $\phi$ 29 DNA polymerase.